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THE MUTAGENIC POTENTIAL OF: N,N-DIPROPYLCYCLOHEXANECARBOXIMIDE --ETC(U)
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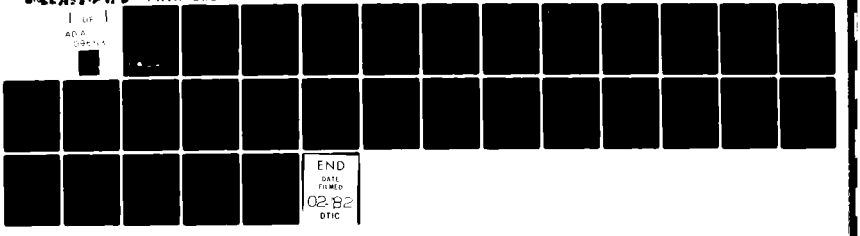

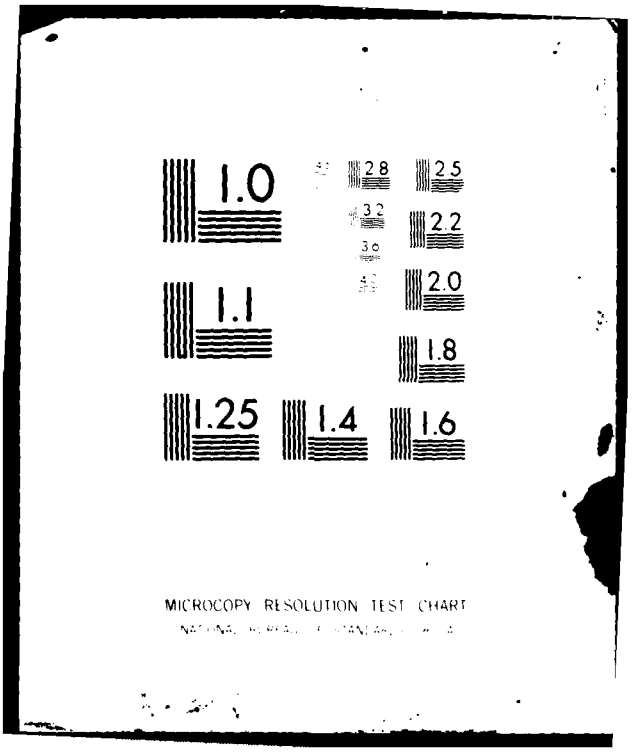
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AD A109653

INSTITUTE REPORT NO. 108

THE MUTAGENIC POTENTIAL OF:

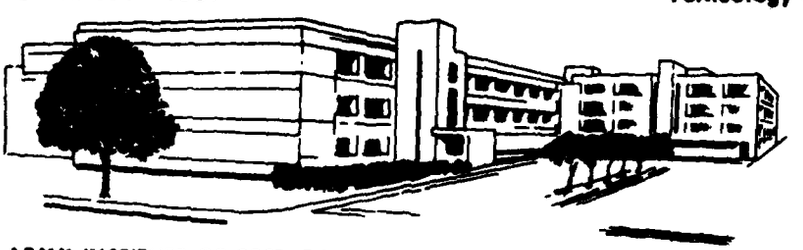
***N,N*-dipropylcyclohexanecarboxamide (CHR 10)**
1-(3-cyclohexene-1-yl-carbonyl) piperidine (CHR 11)

LEONARD J. SAUERS, BA, SP5
and
JOHN T. FRUIN, DVM, PhD, LTC VC

TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT

NOVEMBER 1981

Toxicology Series -23



LETTERMAN ARMY INSTITUTE OF RESEARCH PRESIDIO OF SAN FRANCISCO CALIFORNIA 94129

Toxicology Series: 23

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30 Nov 1981

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The mutagenic potential of N,N-dipropylcyclohexanecarboximide ~~(CHR 10)~~ and 1-(3-cyclohexene-1-yl-carbonyl)piperidine ~~(CHR 11)~~ was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 were exposed to doses ranging from 1 ul/plate to 6.2×10^{-4} ul/plate. It was determined that none of the tested substances had mutagenic potential.

*Code number for compound.

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ABSTRACT

The mutagenic potential of N,N-dipropylcyclohexanecarboximide (CHR 10)* and 1-(3-cyclohexene-1-yl-carbonyl) piperidine (CHR 11)* was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 were exposed to doses ranging from 1 ul/plate to 3.2×10^{-4} ul/plate. It was determined that none of the tested substances had mutagenic potential.

* Code number for compound.

PREFACE

	Substance	Code No.
AMES ASSAY REPORT:	N,N,-dipropylcyclohexanecarboximide	CHR 10
	1-(3-cyclohexene-1-yl-carbonyl) piperidine	CHR 11

TESTING FACILITY: Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

SPONSOR: Division of Cutaneous Hazards
Letterman Army Institute of Research

PROJECT: More Effective Topical Repellents Against Disease Bearing Mosquitoes 3M62272A810

GLP STUDY NUMBER: 81029

STUDY DIRECTOR: LTC (P) John T. Fruin, DVM, PhD, VC, Diplomate of American College of Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: SP5 Leonard J. Sauers, BA

RAW DATA: A copy of the final report, study protocol, and retired SOPs will be retained in the LAIR Archives. Test chemicals were provided by the sponsor. Our information about the chemical analysis of the two test compounds was obtained from McGovern (Appendix A).

PURPOSE: To determine the mutagenic potential of CHR 10 and CHR 11 by using the Ames Salmonella/Mammalian Microsome Mutagenicity Test. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were used.

ACKNOWLEDGMENT

The authors wish to thank John Dacey, SP4 Lawrence Mullen, BS, and SP4 Thomas Kellner, BA for their assistance in performing the research.

Signatures of Principal Scientists Involved
In The Study

We, the undersigned, believe the study number 81029 described in this report to be scientifically sound and the results in this report and interpretation to be valid. The study was conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations for Non-Clinical Laboratory Studies, outlined by the Food and Drug Administration.

Leonard J. Sauers 11/23/81

LEONARD J. SAUERS/DATE
SP5, BA
Principal Investigator

John T Fruin 24 Nov 81

JOHN T. FRUIN, DVM, PhD/DATE
LTC (P), VC
Study Director

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

23 November 1981

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 81029 the following inspections were made:

22 Sep 81
24 Sep 81
2 Oct 81
17 Nov 81

Inspection findings were reported to the Study Director on 24 Sep 81. Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the Oct and Dec report to management and the Study Director.

JOHN C. JOHNSON
CPT, MS
Quality Assurance Officer

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The insect repellent program is directed to the development of better insect repellents for the protection of soldiers from insects and insect-borne diseases in the field. In the last several years the Letterman Army Institute of Research (LAIR) Division of Cutaneous Hazards has tested a large number of chemical compounds submitted by the SRI International, the U.S. Department of Agriculture (USDA) and private industry against a variety of mosquitoes, sand flies, fleas, bugs, ticks and mites in animals and in vitro test systems. Several of these materials have shown sufficient repellent activity and persistence on the skin of animals to warrant consideration for use in lieu of or in conjunction with the current troop-issue insect repellent, 75% N,N-diethyl-m-toluamide (m-DEET) in ethanol. The Division of Cutaneous Hazards has also evaluated a number of new formulations of m-DEET prepared at LAIR or submitted by private industry. Several of these new formulations have been more persistent than the current troop-issue repellent in tests on animals.

It is now planned to test the best of the new compounds and formulations on human volunteers to confirm the results that have been obtained in the in vitro and animal tests and to evaluate their performance under conditions of actual use. Before this can be done, it is necessary to obtain certain toxicity data on each compound or formulation to insure that it is safe for application to the skin. The toxicity tests required for registration of a new insect repellent are prescribed by the Environmental Protection Agency (EPA). The basic toxicity tests required for experimental use of the new compounds and formulations on human volunteers are prescribed by the LAIR and USAMRDC Human Use Committees. If adverse toxicity data are obtained in these tests, the respective materials(s) will be eliminated from consideration, and the prospective tests on human volunteers will not be carried out. The toxicity testing program thereby serves as both a safety factor and secondary screen in the repellent development scheme.

Rationale for using the Ames Assay

The Ames Salmonella/Mammalian Microsome Mutagenicity Test is one of a standard bank of tests used by our laboratory for the assessment of the mutagenic potential of a test substance. It is a short-term screening assay, which we use for the prediction of potential mutagenic agents in mammals. It is inexpensive when compared to in vivo tests, yet is highly predictive and reliable in its ability to detect mutagenic activity and therefore carcinogenic probability (1). It relies on basic genetic principles and allows for the incorporation of a mammalian microsome enzyme system to increase sensitivity through enzymatically altering the test substance into an active metabolite. It has proven highly effective in assessing human risk (1).

Description of Test (Rationale for the selection of strains)

The test was developed by Bruce Ames, Ph.D. from the University of California-Berkeley. The test involves the use of several different genetically altered strains of Salmonella typhimurium, each with a specific mutation in the histidine operon (2). The test substance demonstrates mutagenic potential if it is able to revert the mutation in the bacterial histidine operon back to the wild type and thus reestablish prototrophic growth within the test strain. This reversion also can occur spontaneously due to a random mutational event. If, after adding a test substance, the number of revertants is significantly greater than the spontaneous reversion rate, then the test substance physically altered the locus involved in the operon's mutation and is able to induce point mutations and genetic damage (2).

In order to increase the sensitivity of the test system, two other mutations in the Salmonella are used (2). To insure a higher probability of uptake of test substance, the genome for the lipopolysacchride layer (LP) is mutated and allows larger molecules to enter the bacteria. Each strain has another induced mutation which causes loss of excision repair mechanisms. Since many chemicals are not by themselves mutagenic but have to be activated by an enzymatic process, a mammalian microsome system is incorporated. These microsomal enzymes are obtained from livers of rats induced with Aroclor 1254; the enzymes allow for the expression of the metabolites in the mammalian system. This activated rat liver microsomal enzyme homogenate is termed S-9.

Description of Strains (History of the strains used method to monitor the integrity of the organisms, and data pertaining to current and historical control and spontaneous reversion rates)

The test consists of using five different strains of Salmonella typhimurium that are unable to grow in absence of histidine because of a specific mutation in the histidine operon. This histidine requirement is verified by attempting to grow the tester strains on minimal glucose agar (MGA) plates, both with and without histidine. The dependence on this amino acid is shown when growth occurs only in its presence. The plasmids in strains TA 98 and TA 100 contain an ampicillin resistant R factor. Strains deficient in this plasmid demonstrate a zone of inhibition around an ampicillin impregnated disc. The alteration of the LP layer allows uptake by the Salmonella of larger molecules. If a crystal violet impregnated disc is placed onto a plate containing any one of the bacterial strains, a zone of growth inhibition will occur because the LP layer is altered. The absence of excision repair mechanisms can be determined by using ultraviolet (UV) light. These mechanisms function primarily by repairing photodimers between pyrimidine bases; exposure of bacteria to UV light will activate the formation of these dimers and cause cell

lethality, since excision of these photodimers can not be made. The genetic mutation resulting in UV sensitivity also induces a dependence by the Salmonella to biotin. Therefore, this vitamin must be added. In order to prove that the bacteria are responsive to the mutation process, positive controls are run with known mutagens. If after exposure to the positive control substance, a larger number of revertants are obtained, then the bacteria is adequately responsive. Sterility controls are performed to determine the presence of contamination. Sterility of the test compound is also confirmed in each first dilution. Verification of the tester strains occurs spontaneously with the running of each assay. The value of the spontaneous reversion rate is obtained by using the same inoculum of bacteria that is used in the assay (3).

Strains were obtained directly from Dr. Ames, University of California-Berkeley, propagated and then maintained at -80 C in our laboratory. Before any substance was tested, quality controls were run on the bacterial strains to establish the validity of their special features and also to determine the spontaneous reversion rate (2). Records are maintained of all the data to determine if deviations from the set trends have occurred.

In this series of tests for the detection of mutagenic potential of different agents, we compare the spontaneous reversion values with our own historical values and these cited by Ames et al (2). Our conclusions are based on the spontaneous reversion rate compared to the experimentally induced rate of mutation. When operating effectively, these strains detect substances that cause base pair mutations (TA 1535, TA 100) and frameshift mutations (TA 1537, TA 1538, and TA 98).

METHODS (3)

Rationale for Dosage Levels and Dose Response Tabulations

To insure readable and reliable results, a sublethal concentration of the test substance had to be determined. This toxicity level was found by using MGA plates, various concentrations of the substance, and approximately 10^8 cells of TA 100 per plate, unless otherwise specified. Top agar containing trace amounts of histidine and biotin were placed on MGA plates. TA 100 is used because it is the most sensitive strain. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth was observed on the plates. (The auxotrophic Salmonella will replicate a few times and potentially express a mutation. When the histidine and biotin supplies are exhausted, only those bacteria that reverted to the prototrophic phenotype will continue to reproduce and form macrocolonies; the remainder of the bacteria comprises the background

lawn. The minimum toxic level is defined as the lowest serial dilution at which decreased macrocolony formation, below that of the spontaneous revertant rate, and an observable reduction in the density of the background lawn occurs.) A maximum dose of 1 mg/plate is used when no toxicity is observed. The densities were recorded as normal slight, and no growth.

Test Format

After we validated our bacterial strains and determined the optimal dosage of the test substance, we began the Ames Assay. In the actual experiment, 0.1 ml of the particular strain of Salmonella (10^8 cells) and the specific dilutions of the test substance are added to 2 ml of molten top agar, which contained trace amounts of histidine and biotin. Since survival is better from cultures which have just passed the log phase, the Salmonella strains are used 16 hours (maximum) after initial inoculation into nutrient broth. The dose of the test substance spanned a 1000-fold, decreasing from the minimum toxic level by a dilution factor of 5. All the substances were tested with and without S-9 microsome fraction. The optimal titer of the S-9 was determined and 0.5 ml was added to the molten top agar. After all the ingredients were added, the top agar was mixed, then overlaid on minimum glucose agar plates. These plates contained 2% glucose and Vogel Bonner "E" Concentrate (4). The water used in this medium and all reagents came from a polymetric system. Plates were incubated, upside down in the dark at 37 C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The corresponding number of revertants obtained was compared to the number of spontaneous revertants; the conclusions were recorded statistically. A correlated dose response is considered necessary to declare a substance as a mutagen. Commoner (5), in his report, "Reliability of Bacterial Mutagenesis Techniques to Distinguish Carcinogenic and Non-Carcinogenic Chemical," and McCann et al (1) in their paper, "Detection of Carcinogens as Mutagen: Assay of over 300 Chemicals," have concurred on the test's ability to detect mutagenic potential.

Statistical Analysis

Quantitative evaluation was ascertained by two independent methods. Ames et al (2) assumed that a compound which caused twice the spontaneous reversion rate is mutagenic. Commoner (5), developed the MUTAR Ratio, which is stated in the following equation:

$$MUTAR = (E - C)/C_{AV}$$

Here, C is the number of spontaneous revertant colonies on control plates obtained on the same day and with the same treatment and strains. E is the number of revertants in response to the compound;

C_{AV} is the number of spontaneous revertants on control plates calculated from historical records. The explanation of the results of this equation can be determined by the method of Commoner (5). This variation determines the probability of correctly classifying substances as carcinogens on the basis of their mutagenic activity. The E values were recorded by strain, with and without S-9. Values for C and C_{AV} were recorded separately.

We used the formula and logged all values for our permanent records.

Chemical Analysis

Our information about the chemical analysis of the two test compounds was obtained from McGovern (Appendix A).

RESULTS AND DISCUSSION

Throughout this report, all test compounds will be referred to by their respective code number:

Substance	Code No.
N,N,-dipropylcyclohexanecarboximide	CHR 10
1-(3-cyclohexene-1-yl-carbonyl) piperidine	CHR 11

On 18 September 1981, the toxicity level determination was run on the two test substances. All sterility and positive controls were normal. The spontaneous reversion rate for TA 100 was also as expected (Table 1). Toxic responses were observed for both compounds at the initial dose of 10 ul/plate (Table 2A-2B). It was decided to use 1 ul/plate as the initial dose for the Ames Assay.

On 22 September 1981, the Ames Test was performed on the two test substances. All sterility and strain verification controls were normal (Table 3). All positive controls were normal except the response of TA 98 and TA 100 to dimethyl benzanthracene (DMBA). These tester strains did react as expected to all other positive controls. The spontaneous reversion rates were all within normal limits (Table 4).

No evidence of mutagenic potential was observed in response to CHR 10 (Table 5A). There was only one isolated instance of a doubling of the spontaneous reversion rate in response to CHR 11. This occurred at the 0.0016 ul/plate dose for activated TA 1535. No dose response was observed (Table 5B). The MUTAR values listed in Tables 6A-6B were all normal.

CONCLUSION

On the basis of the Ames Assay, Compounds CHR 10 and CHR 11 are not mutagenic at the levels tested.

RECOMMENDATION

CHR 10 and CHR 11 should be tested by using other toxicological assays if efficacy tests prove these compounds to be promising repellents.

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4. VOGEL, H. J. and D. M. BONNER. Acetylornithinase of E. coli: Partial purification and same properties, J Biol Chem, 218: 97-106, 1956

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Letter, Information about

N,N-dipropylcyclohexanecarboximide and
1-(3-cyclohexene-1-yl-carbonyl) piperidine

APPENDIX A

United States Department of Agriculture
Science and Education Administration
Agricultural Research Northeastern Region Beltsville Agricultural Research Center
Beltsville Maryland 20705

October 16, 1981

Dr. J. T. Fruin, Chief
Toxicology Group
Department of the Army
Letterman Army Institute of Research
Presidio of San Francisco, California 94129

Dear Dr. Fruin:

Information requested of me in your letter of October 6, 1981 concerning N,N-dipropylcyclohexanecarboxamide and 1-(3-cyclohexene-1-yl carbonyl)piperidine is as follows:

a) the compounds are amides and are very stable under ordinary conditions;

b) I do not know the purity of the samples you have on hand because I did not supply them to Mr. Rutledge, however, if they were obtained from USAEHA, Aberdeen, Maryland, they are of high Purity (>99%);

c) purity was determined by gc analysis on 6' x 1/8" SS columns packed with 3% SE-30 on Varaport 30, 100/120 mesh and 3% OV101 on Gas Chrom Q, 100/120 mesh;

d) we have not determined the % solubility in various solvents but, in general, they are soluble in polar solvents.

I hope this information will be of use to you.

Sincerely,

T. P. McGovern

TERRENCE P. MCGOVERN, Research Chemist
Organic Chemical Synthesis Laboratory
Agricultural Environmental Quality Institute

cc:
J. R. Plimmer
M. Weeks

APPENDIX A

LIST OF TABLES

APPENDIX B

Table 1

STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION

Strains	Histidine Requirement	Ampicillin Resistance	UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
100	NG	G	NG	13.89 mm	NG	+
1537	NG	14.11 mm	NG	12.66	NG	+
WT	G	NA	G	NA	NG	+

STERILITY CONTROL

His-Bio Mix Initial: NG End: NG MGA Plate: NG

Top Agar Initial: NG End: NG

Diluent: NG Nutrient Broth: NG

Test Compound (a) CHR10-NG (b) CHR11-NG (c) NA (d) NA (e) NA

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

Spontaneous Revertants: TA 100, No S-9 74,55,70,63,69,66 av. 66

(1) + = expected response - = unexpected response

Study Number: 81029 Date: 18 Sep 81 By: Sauers, Dacey, Mullen

Table 2A

TOXICITY LEVEL DETERMINATION

Substance assayed: CHR 10 Substance dissolved in: ETOH

Study Number: 81029 Date: 18 Sep 81 Performed by: Sauers, Dacey, Mullen

TA 100 REVERTANT PLATE COUNT

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn (1)
10 ul/plate	Toxic	Toxic	Toxic	Toxic	NG
1 ul/plate	60	80	62	67	NL
10^{-1} ul/plate	80	71	67	73	NL
10^{-2} ul/plate	63	81	58	67	NL
10^{-3} ul/plate	80	65	61	69	NL
10^{-4} ul/plate	87	60	68	72	NL
10^{-5} ul/plate	71	96	86	84	NL
10^{-6} ul/plate	67	83	71	74	NL

(1) NG = No Growth ST = Slight Growth NL = Normal Lawn

Table 2B

TOXICITY LEVEL DETERMINATION

Substance assayed: CHR 11 Substance dissolved in: ETOH

Study Number: 81029 Date: 18 Sep 81 Performed by: Sauers, Mullen, Dacey

TA 100 REVERTANT PLATE COUNT

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn (1)
10 ul/plate	TOXIC	TOXIC	TOXIC	TOXIC	NG
1 ug/plate	68	60	74	67	NL
10^{-1} ul/plate	70	60	73	68	NL
10^{-2} ul/plate	79	90	64	78	NL
10^{-3} ul/plate	60	62	70	64	NL
10^{-4} ul/plate	63	60	57	60	NL
10^{-5} ul/plate	56	53	72	60	NL
10^{-6} ul/plate	78	50	55	61	NL

(1) NG = No Growth ST = Slight Growth NL = Normal Lawn

Table 3

STRAIN VERIFICATION CONTROL

Strains	Histidine Requirement	Ampicillin Resistance	UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
98	NG	G	NG	15 mm	NG	+
100	NG	G	NG	14 mm	NG	+
1535	NG	NA	NG	16 mm	NG	+
1537	NG	25 mm	NG	14 mm	NG	+
1538	NG	NA	NG	15 mm	NG	+
WT	G	NA	G	NA	G	+

STERILITY CONTROL

His-Bio Mix Initial: NG End: NG Diluent: NG

Top Agar Initial: NG End: NG MGA Plate: NG

S-9 Mix Initial: NG End: NG Nutrient Broth: NG

Test Compound (a) CHR 10-NG (b) CHR 11-NG (c) NA (d) NA (e) NA (f) NA

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

(1) + = expected response
- = unexpected response

Study Number: 81029

By: Sauers, Kellner, Mullen, Dacey

Date: 22 sept 81

Table 4

SPONTANEOUS REVERTANT RATE AND POSITIVE CONTROL REVERTANT RATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
AF	2 ug/plate	yes	(564,510,666) (580)	(340,287,346) (324)			(740,647,614) (667)
BF	2 ug/plate	yes	(101,177,176) (151)	(298,375,323) (332)		(45,51,60) (52)	(89,78,65) (77)
DMBA	20 ug/plate	yes	(56,61,40) (52)	(209,193,205) (202)		(30,27,23) (27)	(61,52,51) (55)
MNNG	2 ug/plate	no		(801,747,440) (663)			
	20 ug/plate	no			(356,333,479) (389)		

Strain Performance

Spontaneous Revertants	S-9 Added	98	100	1535	1537	1538
before	no	(18,22,26)	(162,139,123)	(17,20,20)	(6,4,8)	(17,16,18)
after		(15,28,30) (23)	(125,95,134) (130)	(23,18,21) (20)	(5,2,4) (5)	(13,18,17) (16)
before	yes	(40,29,24)	(129,111,158)	(12,14,10)	(8,8,5)	(22,19,17)
after		(27,27,19) (28)	(152,134,115) (133)	(15,17,17) (14)	(4,6,8) (6)	(28,27,15) (21)

Study Number: 81029

Date: 22 Sep 81 By: Sauers, Mullen, Kellner, Dacey

Table 5A
NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
CHR 10	1 ul/plate	no	(25,23,21) (23)	(89,102,123) (105)	(23,20,22) (22)	(3,4,3) (3)	(12,15,11) (13)
		yes	(30,33,34) (32)	(138,105,122) (122)	(12,26,24) (21)	(8,3,4) (5)	(17,12,18) (16)
CHR 10	0.2 ul/plate	no	(15,27,18) (20)	(108,107,121) (112)	(15,27,26) (23)	(2,4,8) (5)	(17,14,6) (12)
		yes	(28,44,22) (31)	(93,123,115) (110)	(17,26,21) (21)	(4,6,7) (6)	(14,21,15) (17)
CHR 10	0.04 ul/plate	no	(18,25,27) (23)	(120,115,107) (114)	(29,25,18) (24)	(5,4,3) (4)	(12,16,16) (15)
		yes	(38,23,22) (28)	(102,113,115) (110)	(24,13,19) (19)	(8,6,4) (6)	(20,17,12) (16)

-continued

Study Number: 81029 Date: 22 Sep 81 By: Sauers, Mullen, Kellner, Dacey

Table 5A, concluded

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
CHR 10	0.008 ul/plate	no	(12,16,21) (16)	(89,106,109) (101)	(23,11,18) (17)	(6,5,9) (7)	(15,11,27) (18)
		yes	(31,36,34) (34)	(97,131,93) (107)	(24,21,27) (24)	(4,6,2) (4)	(15,10,8) (11)
	0.0016 ul/plate	no	(23,17,14) (18)	(82,102,110) (98)	(11,10,15) (12)	(3,4,2) (3)	(19,12,6) (12)
		yes	(33,30,27) (30)	(89,83,96) (89)	(15,18,23) (19)	(5,7,4) (5)	(31,21,15) (22)
	0.00032 ul/plate	no	(30,20,18) (23)	(130,96,116) (114)	(17,17,24) (19)	(4,4,5) (4)	(8,19,15) (14)
		yes	(35,15,38) (29)	(89,117,148) (118)	(24,18,32) (25)	(5,9,3) (6)	(Contam. 14,15) (14)

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Table 5B

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
CHR 11	1 ul/plate	no	(27,23,21) (24)	(102,96,127) (108)	(19,25,22) (22)	(4,6,3) (4)	(7,14,9) (10)
		yes	(26,26,24) (25)	(98,96,127) (107)	(10,18,22) (17)	(4,3,8) (5)	(23,17,16) (19)
CHR 11	0.2 ul/plate	no	(24,26,17) (22)	(96,100,113) (103)	(16,10,13) (13)	(4,3,5) (4)	(7,15,11) (11)
		yes	(31,35,31) (32)	(114,96,128) (113)	(20,15,32) (22)	(8,3,4) (5)	(23,17,25) (22)
CHR 11	0.04 ul/plate	no	(35,18,28) (27)	(101,122,119) (114)	(13,14,25) (17)	(3,2,9) (5)	(17,14,20) (17)
		yes	(23,29,42) (31)	(92,84,105) (94)	(15,27,18) (20)	(3,7,4) (5)	(35,18,23) (25)

-continued

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Table 5B, concluded

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
CHR 11	0.008 ul/plate	no	(14,11,20) (15)	(116,102,102) (107)	(25,17,24) (22)	(5,6,4) (5)	(14,11,26) (17)
		yes	(36,27,26) (30)	(114,132,111) (119)	(18,14,4) (12)	(4,6,7) (6)	(24,17,20) (20)
CHR 11	0.0016 ul/plate	no	(22,21,26) (23)	(102,105,109) (105)	(21,18,18) (19)	(11,4,5) (7)	(17,18,12) (16)
		yes	(35,24,36) (32)	(137,134,119) (130)	(26,42,24) (31)	(9,9,4) (7)	(30,17,21) (23)
CHR 11	0.00032 ul/plate	no	(11,27,18) (19)	(98,104,104) (102)	(21,23,20) (21)	(7,9,4) (7)	(11,13,13) (12)
		yes	(30,39,27) (32)	(126,103,102) (110)	(21,19,16) (19)	(7,9,5) (7)	(14,24,27) (22)

Study Number: 81029 Date: 22 Sep 81 By: Sauers, Mullen, Kellner, Dacey

Table 6A

MUTAGENIC ACTIVITY RATIO

Substance Assayed: CHR 10 Dissolved in: ETOH

Study Number: 81029 Date: 24 Sep 81 By: Sauers

Concentration	Strain	MUTAR (act)	MUTAR	Concentration	Strain	MUTAR (act)	MUTAR
1 ul/pl	TA 98	0.17	*	0.008 ul/pl	TA 1535	0.86	*
0.2 ul/pl	TA 98	0.12	*	0.0016 ul/pl	TA 1535	0.43	*
0.04 ul/pl	TA 98	*	*	0.00032 ul/pl	TA 1535	0.94	*
0.008 ul/pl	TA 98	0.25	*				
0.0016 ul/pl	TA 98	0.08	*	1 ul/pl	TA 1537	*	*
0.00032 ul/pl	TA 98	0.04	*	0.2 ul/pl	TA 1537	*	*
				0.04 ul/pl	TA 1537	*	*
1 ul/pl	TA 100	*	*	0.008 ul/pl	TA 1537	*	0.34
0.2 ul/pl	TA 100	*	*	0.0016 ul/pl	TA 1527	*	*
0.04 ul/pl	TA 100	*	*	0.00032 ul/pl	TA 1537	*	*
0.008 ul/pl	TA 100	*	*				
0.0016 ul/pl	TA 100	*	*	1 ul/pl	TA 1538	*	*
0.00032 ul/pl	TA 100	*	*	0.2 ul/pl	TA 1538	*	*
				0.04 ul/pl	TA 1538	*	*
1 ul/pl	TA 1535	0.6	0.13	0.008 ul/pl	TA 1538	*	0.15
0.2 ul/pl	TA 1535	0.6	0.19	0.0016 ul/pl	TA 1538	0.05	*
0.04 ul/pl	TA 1535	0.43	0.25	0.00032 ul/pl	TA 1538	*	*

(act): S-9 fraction was added

* : calculated value resulted in a negative MUTAR or zero MUTAR

Table 6B

MUTAGENIC ACTIVITY RATIO

Substance Assayed: CHR 11 Dissolved in: ETOH

Study Number: 81G29 Date: 24 Sep 81 By: Sauers

Concentration	Strain	MUTAR (act)	MUTAR	Concentration	Strain	MUTAR (act)	MUTAR
1 ul/pl	TA 98	*	0.05	0.008 ul/pl	TA 1535	*	0.13
0.2 ul/pl	TA 98	0.17	*	0.0016 ul/pl	TA 1535	1.46	*
0.04 ul/pl	TA 98	0.12	0.2	0.00032 ul/pl	TA 1535	0.43	0.06
0.008 ul/pl	TA 98	0.08	*				
0.0016 ul/pl	TA 98	0.17	*	1 ul/pl	TA 1537	*	*
0.00032 ul/pl	TA 98	0.17	*	0.2 ul/pl	TA 1537	*	*
				0.04 ul/pl	TA 1537	*	*
1 ul/pl	TA 100	*	*	0.08 ul/pl	TA 1537	*	*
0.2 ul/pl	TA 100	*	*	0.0016 ul/pl	TA 1537	0.15	0.34
0.04 ul/pl	TA 100	*	*	0.00032 ul/pl	TA 1537	0.15	0.34
0.008 ul/pl	TA 100	*	*				
0.0016 ul/pl	TA 100	*	*	1 ul/pl	TA 1538	*	*
0.00032 ul/pl	TA 100	*	*	0.2 ul/pl	TA 1538	0.05	*
				0.04 ul/pl	TA 1538	0.22	0.07
1 ul/pl	TA 1535	0.26	0.13	0.008 ul/pl	TA 1538	*	0.07
0.2 ul/pl	TA 1535	0.69	*	0.0016 ul/pl	TA 1538	0.11	*
0.04 ul/pl	TA 1535	0.51	*	0.00032 ul/pl	TA 1538	0.05	*

(act): S-9 fraction was added

* : calculated value resulted in a negative MUTAR or zero MUTAR

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